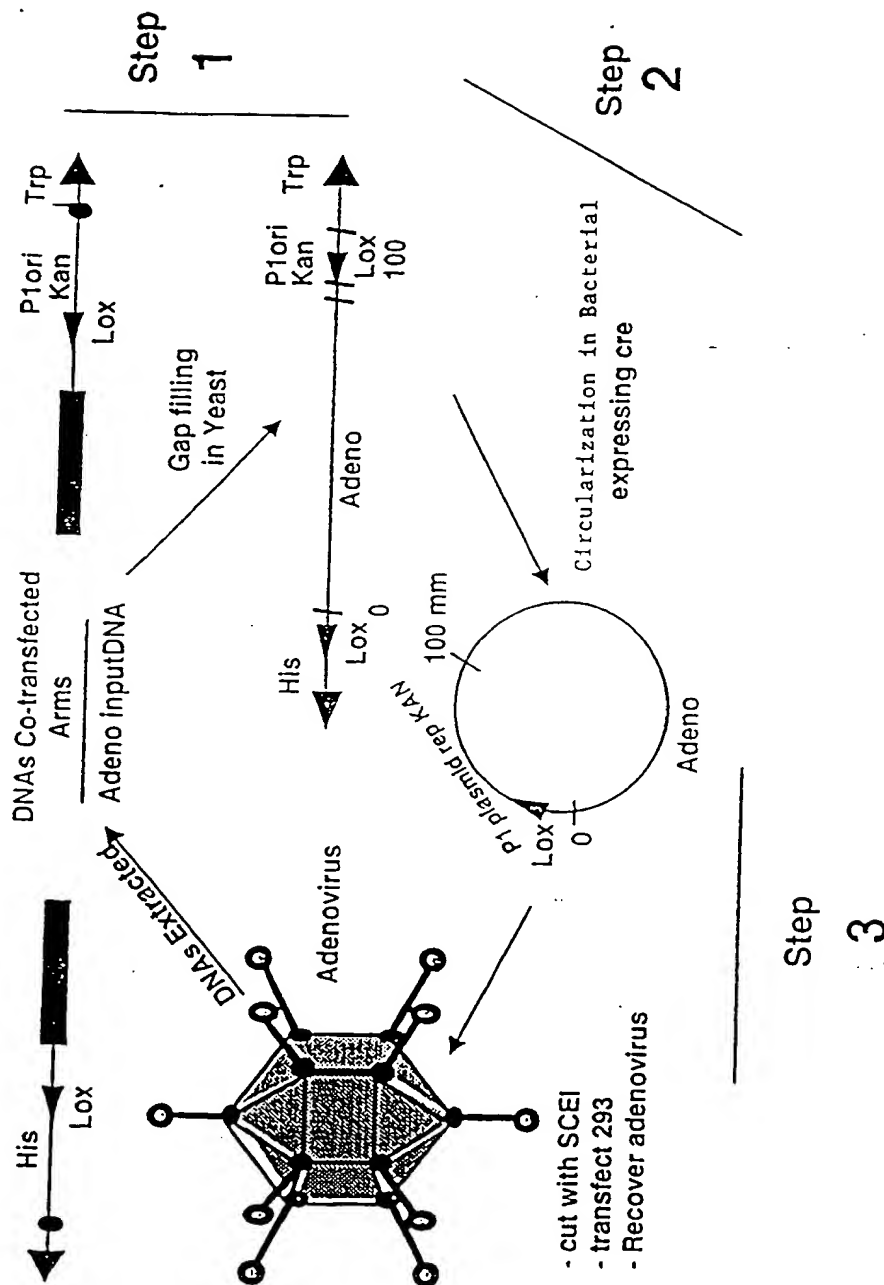


VIRAL CLONING SYSTEM

Figure 1



Circular Viral Cloning System



FOI 2009-0429260

APPROVED	O. G. FIG.
BY	CLASS. CONTROL
DRAFTSMAN	

09/762476

PCT/US00/15588

Figure 3

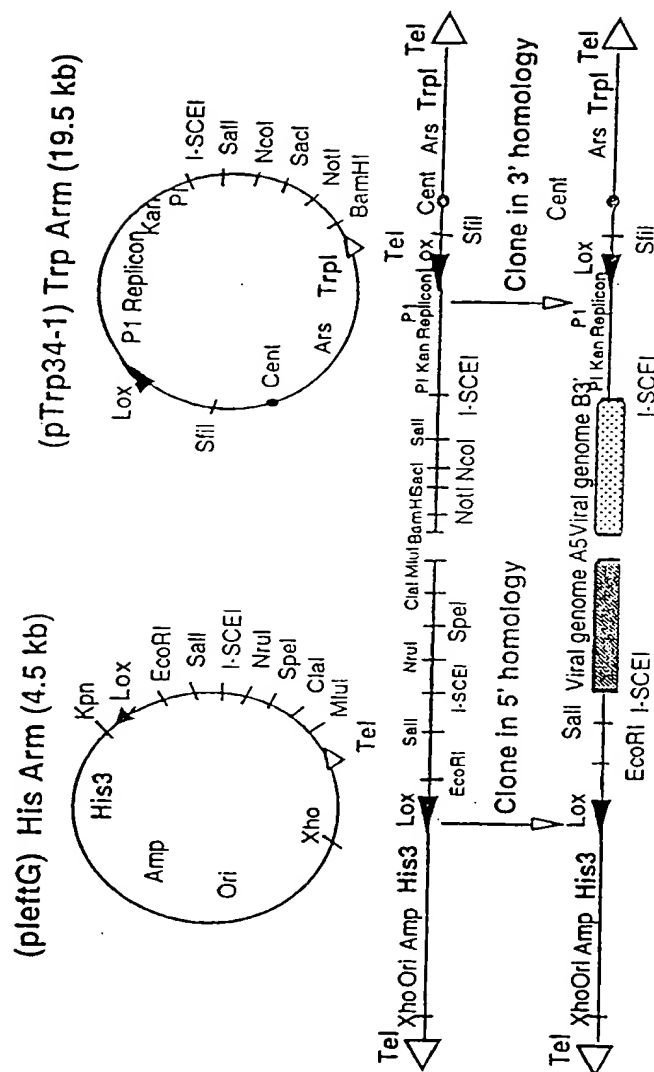
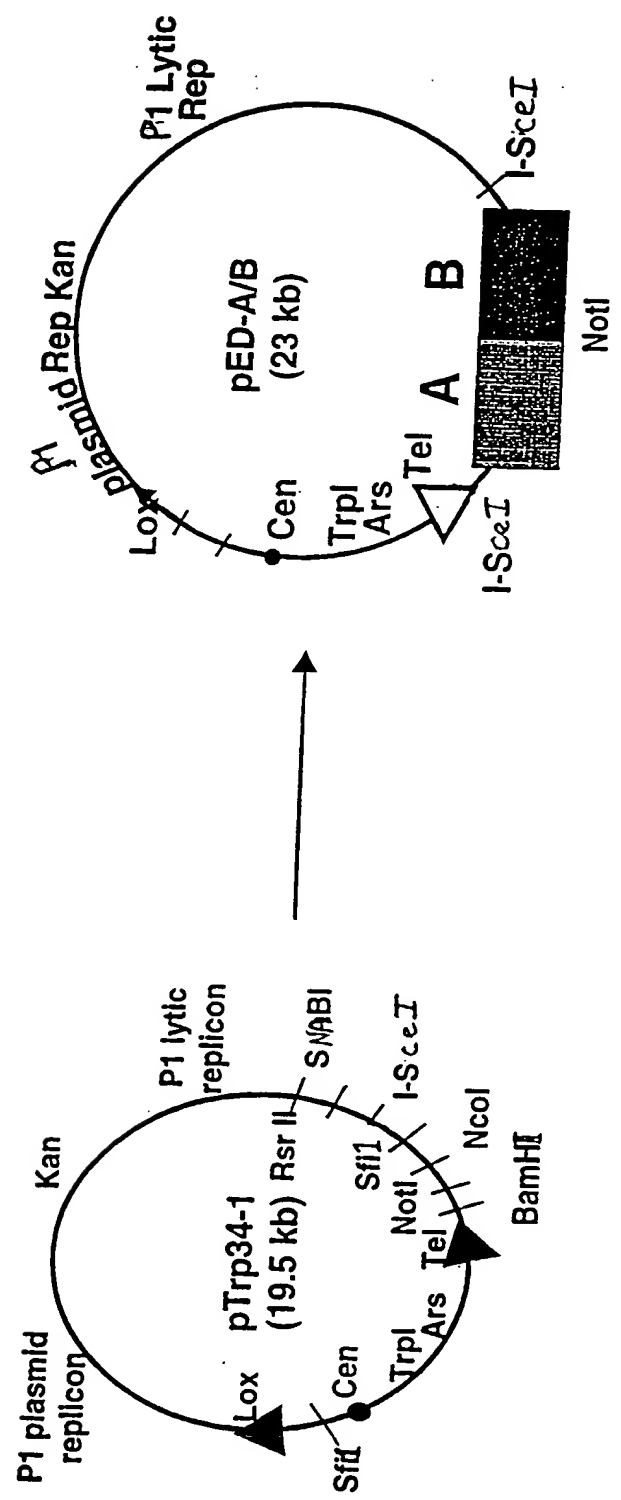


Figure 4



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Figure 5 HIs Arm Construction

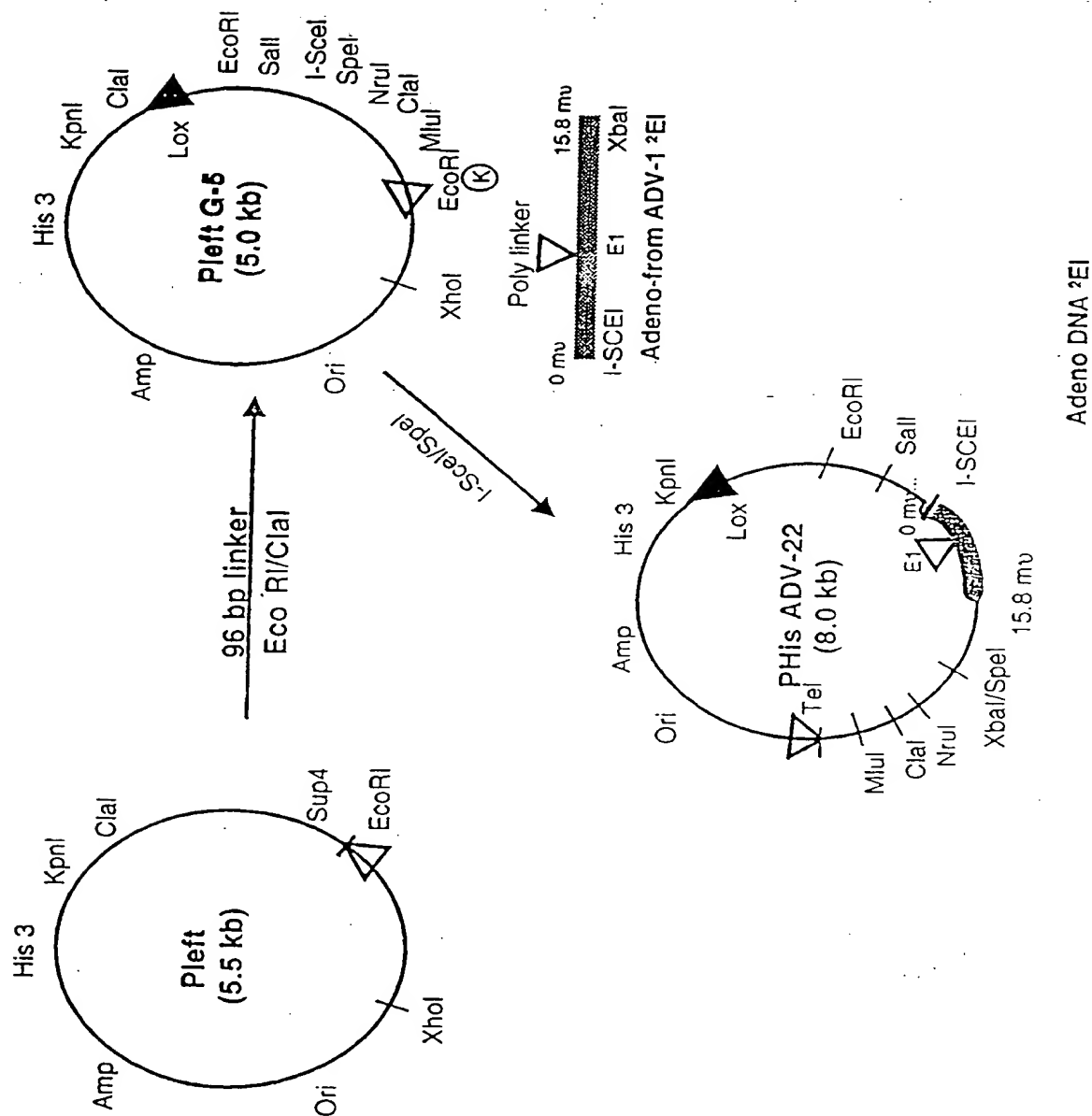
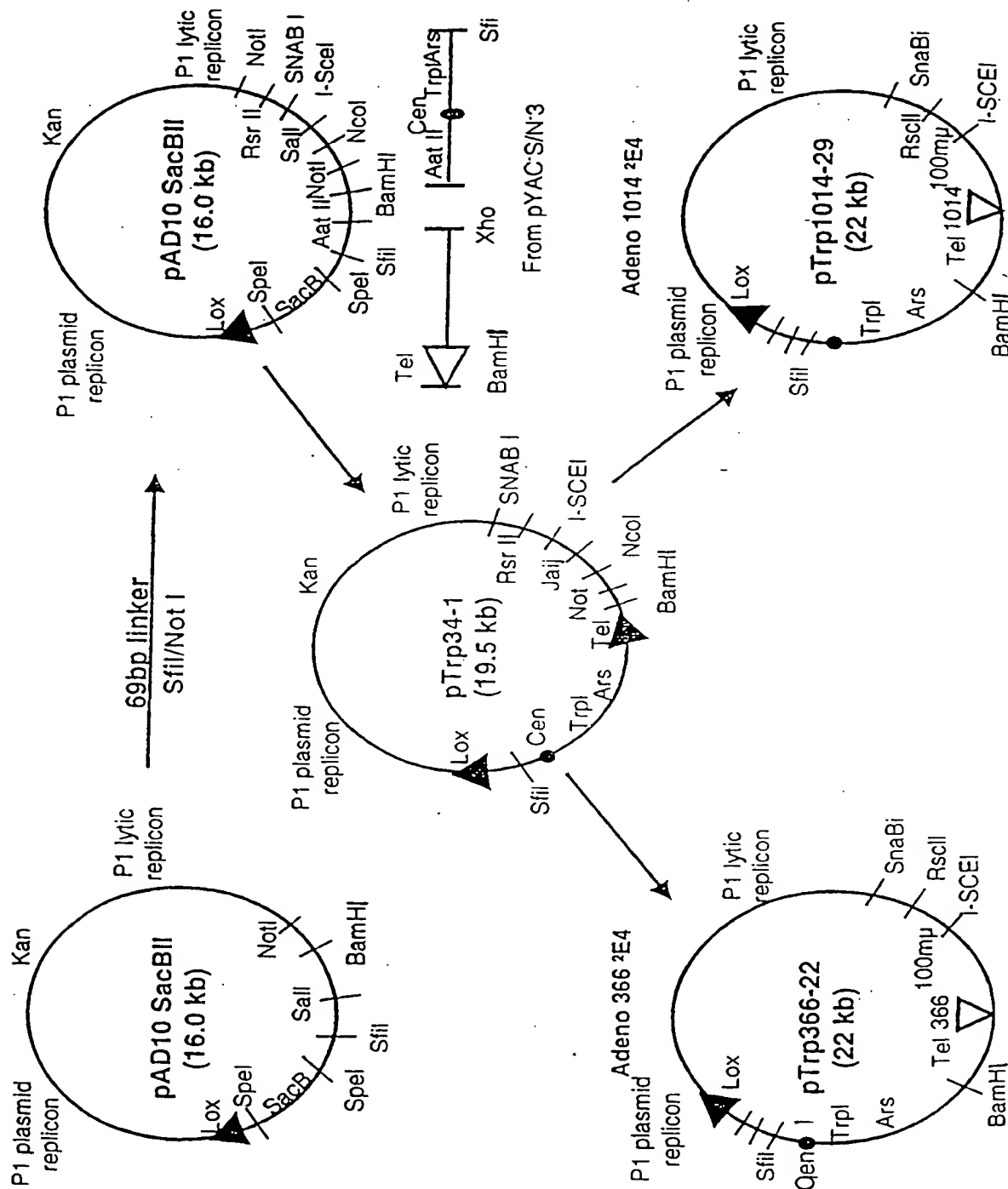
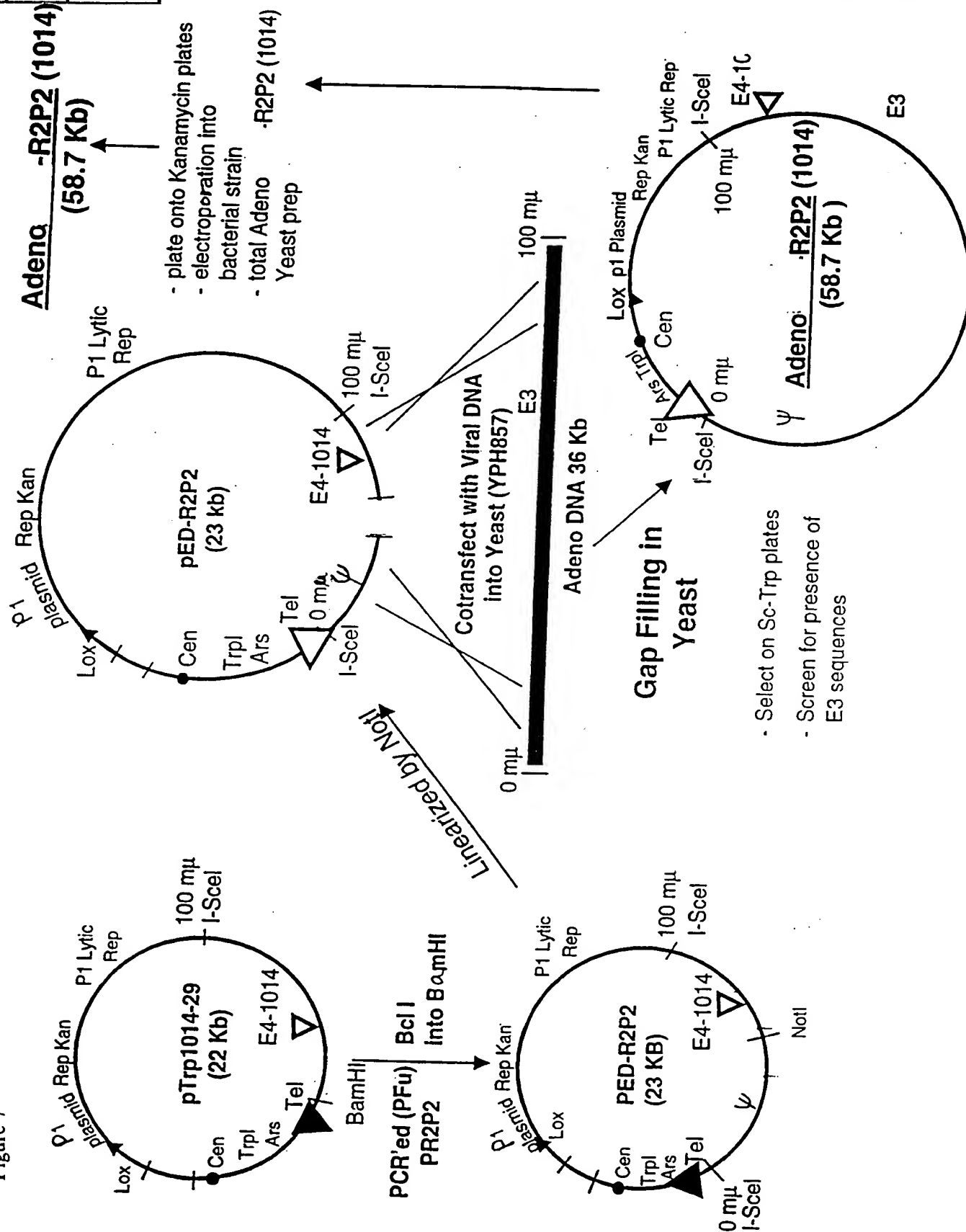
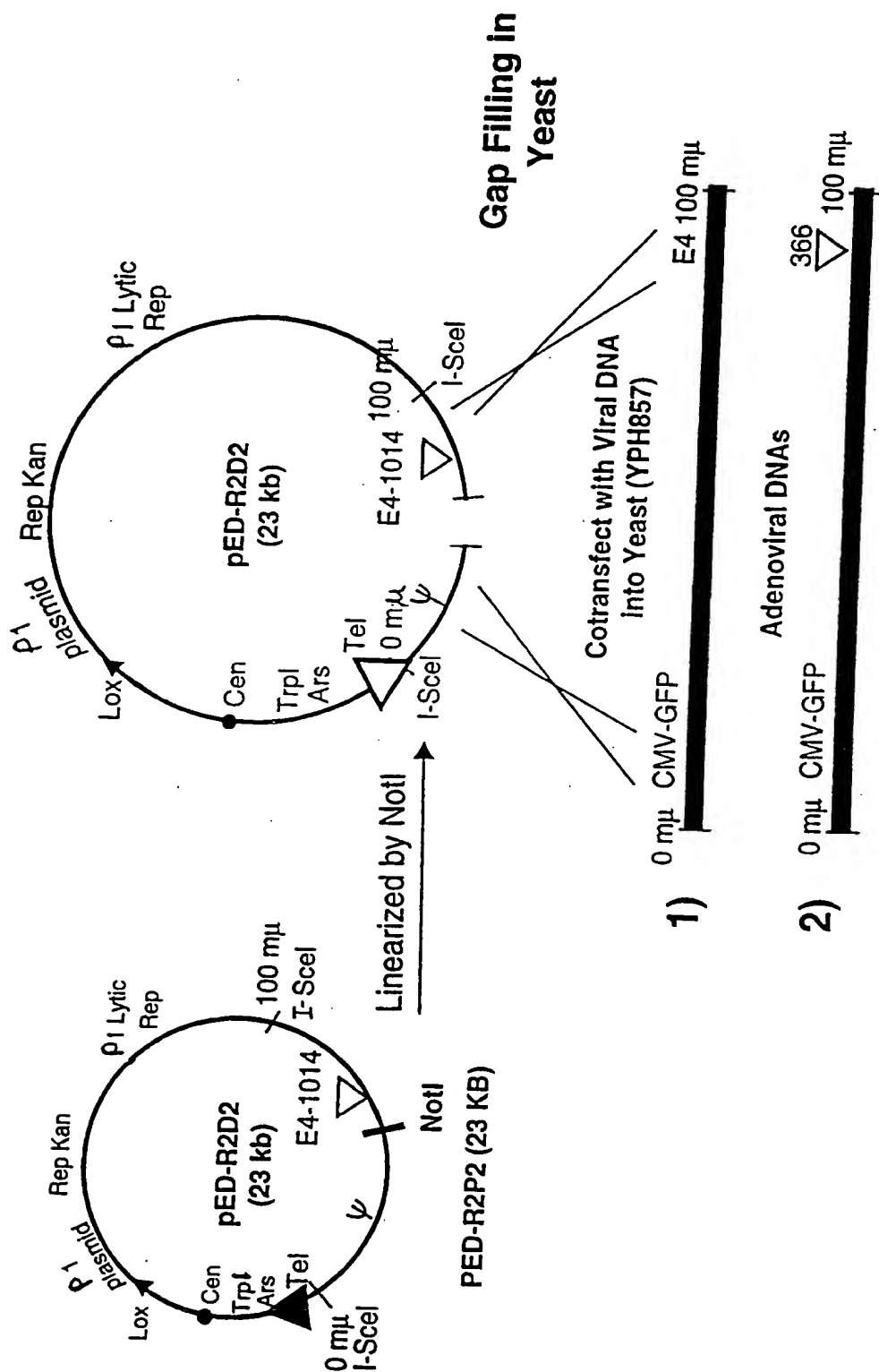


Figure 6 Trp Arm Construction







- Select on Sc-Trp plates
- Screen for presence or absence of orf4 sequences and GFP

Figure 9

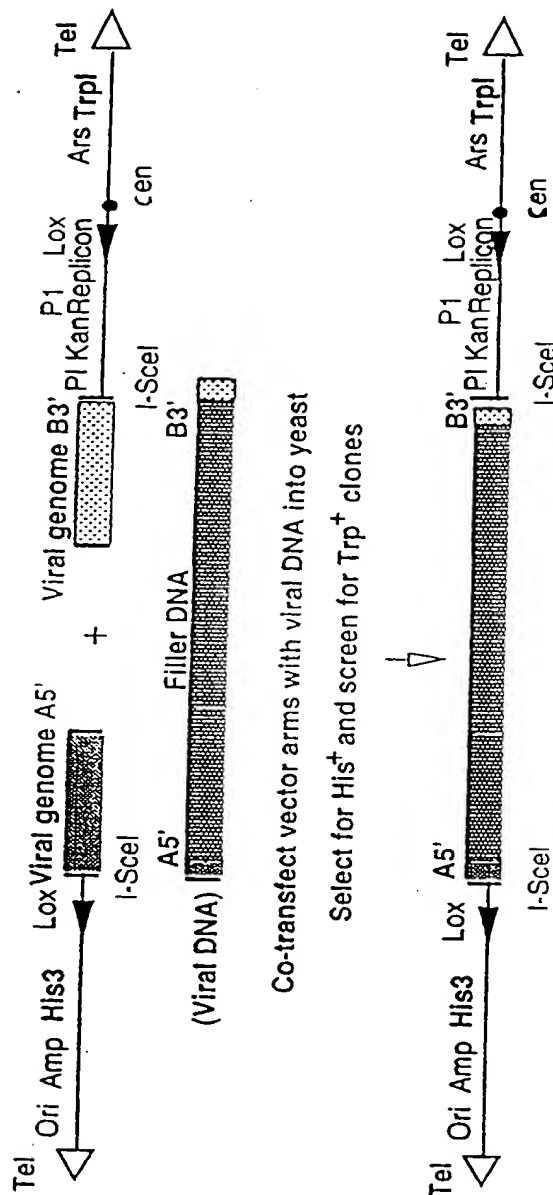
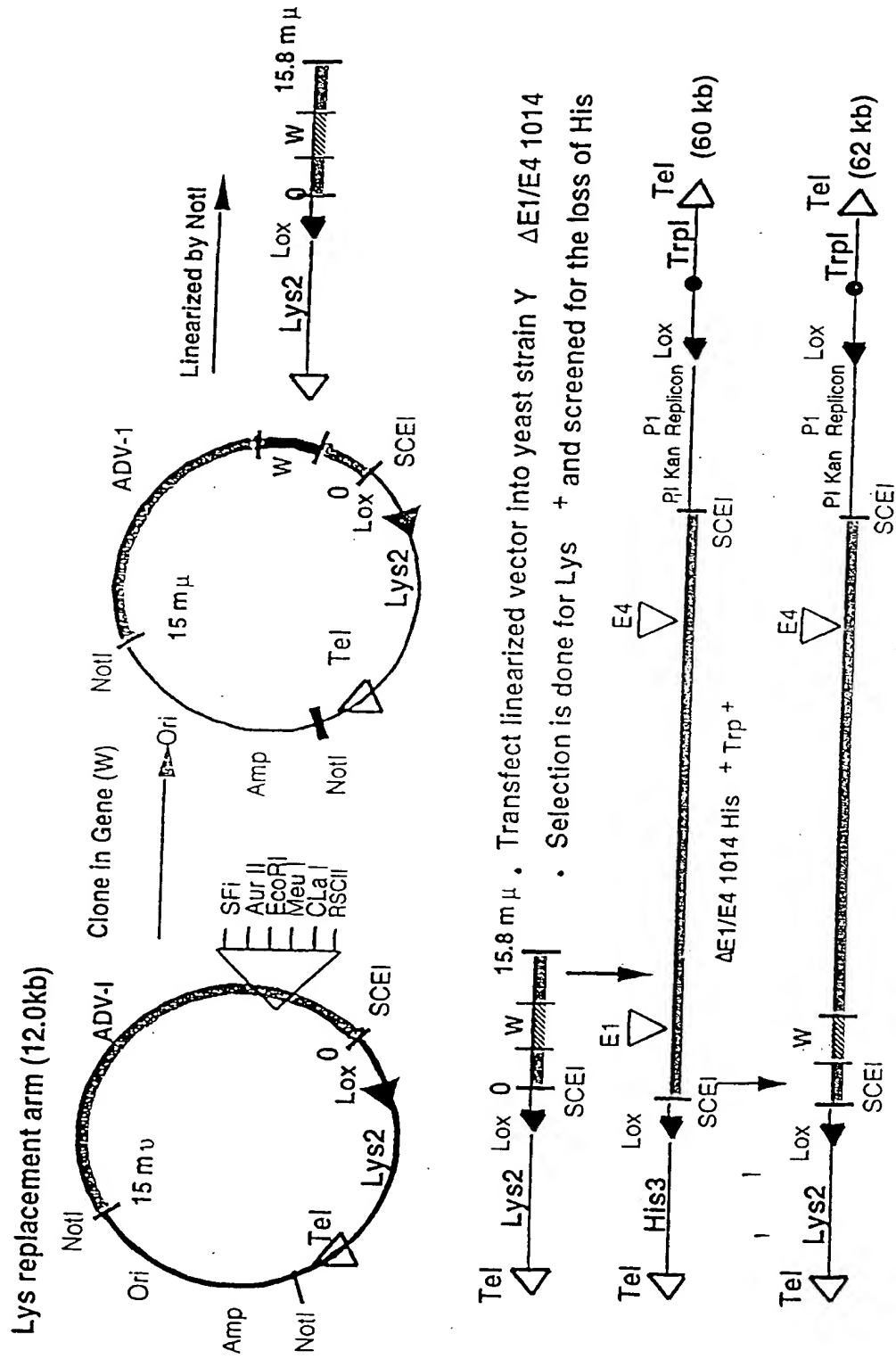


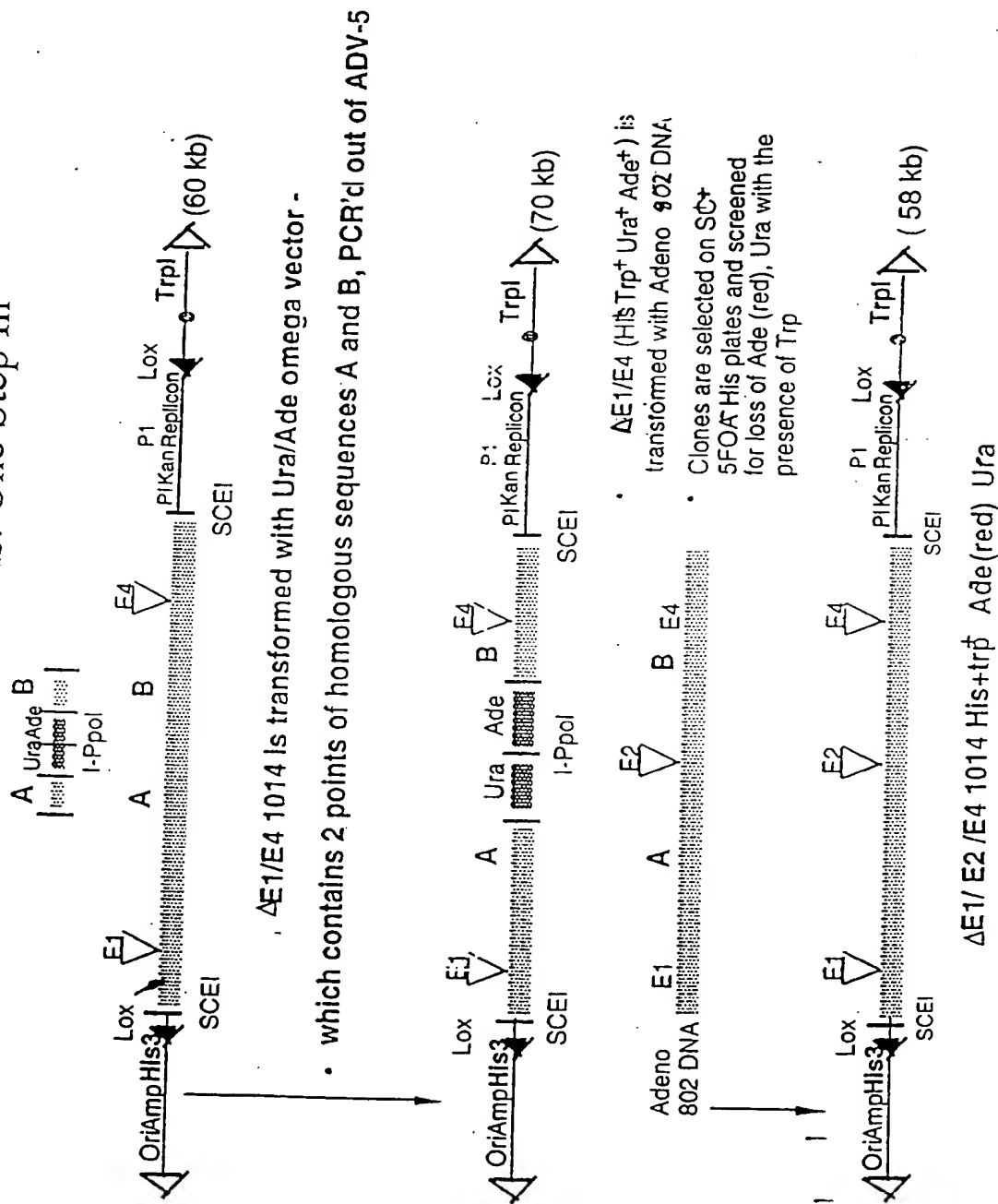


Figure 11
Viral Modifications: Gene Arm Replacement



W ΔE1/E4 1014 Lys + Trp + His⁻

Figure 12 Viral Modifications: One Step In



Ura/Ade

ITR (5 μgs)

ψ

polyAA

- cut input DNA (SfiI)

+

CMV-GFP

E4

Tel

Cen

Ars

TrpI

Kan

Ori

ITR

E4

(10 μgs)

Co-transfect into YPH857

Yeast strain YPH857

AD5-GFP (E4+, E3+)

Trp+ Ade- Ura- (red clones)

Trp(-) Ade(-) Ura(-)

- Lithium acetate

- plate onto SC-Ura(-), Trp(-)

- pick white clones Trp+, Ura(+), Ade(+)

ITR

Ura/Ade

ψ

polyAA

Cen

Tel

E4

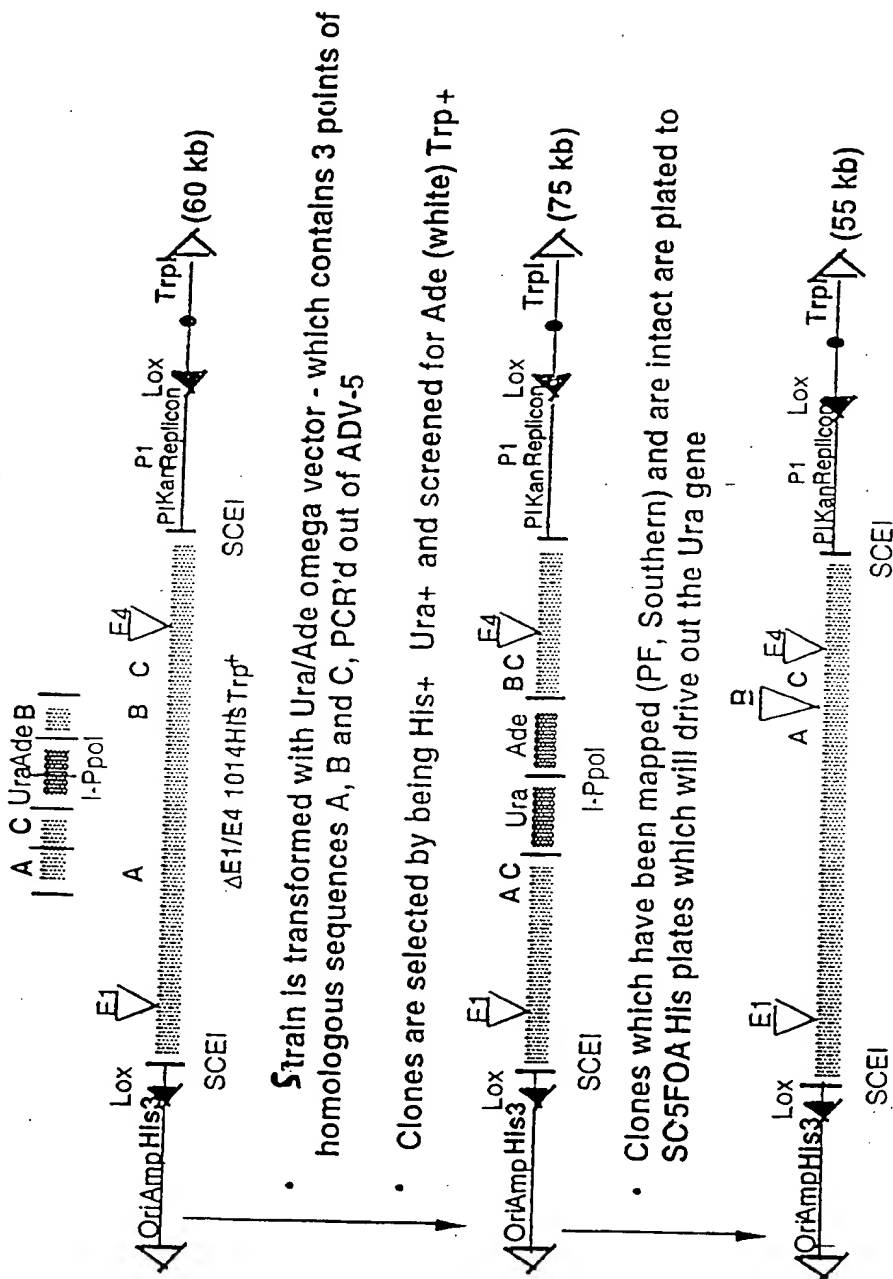
ITR

Ori

* White clones are grown - DNA prepped- cut SmaI

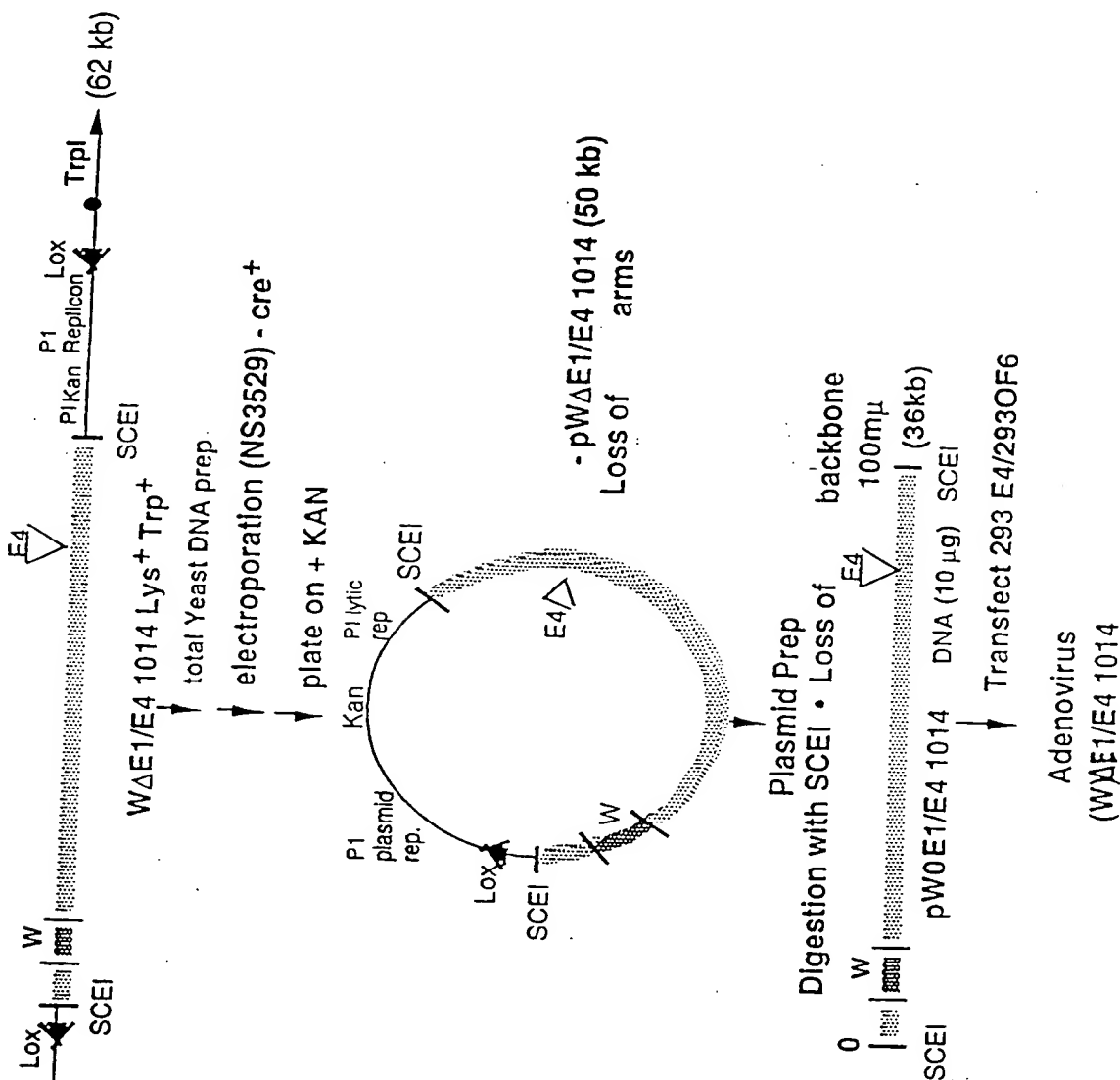
* probe with Ade/Ura and total adeno

Figure 14 Viral Modifications: Two Step In and Out



- Clones would be screened for the loss of Ade (red), Ura and would be His⁺Trp⁺. The loss of Adeno (B) could then be checked for (PF, Southern, PCR)

Figure 15



FD-260 (Rev. 1-7-60)

Figure 16

